ESTIMATING THE ABUNDANCE OF CLUSTERED AND CRYPTIC MARINE MACRO-INVERTEBRATES IN THE GALÁPAGOS WITH PARTICULAR REFERENCE TO SEA CUCUMBERS

By: S. A. Shepherd, V. Toral-Granda, and G. J. Edgar

SUMMARY

Estimating the abundance of marine macro-invertebrates is complicated by a variety of factors: 1) human factors, such as diver efficiency and diver error; and 2) biological factors, such as aggregation of organisms, crypsis, and nocturnal emergence behavior. Diver efficiency varied according to the detectability of an organism causing under-estimation of density by up to 50% in some species. All common species were aggregated at scales from 10-50 m. Transects need to be long enough to transcend the scale of patchiness to improve accuracy. Some species of sea urchins and sea cucumbers (pepinos) which are cryptic by day emerged at night so that daytime censuses underestimated their abundance by up to 10 times. In the sea cucumber fishery, estimates of abundance need to be made at the scale of the population, *i.e.* at hundreds of km. A strategy for this is proposed.

INTRODUCTION

Surveys of fish and marine macro-invertebrates on rocky bottoms are used in landscape ecology to identify bioregions and their subunits. In the Galápagos Islands, surveys are in progress to help classify bioregions for the purpose of zoning the islands according to the types of use permitted. Survey data are also employed to estimate the abundance of the sea cucumber, *Stichopus fuscus*, for the purpose of fixing quotas in the sea cucumber fishery.

The data collected on reef surveys are subject to numerous sources of error. The spatial dispersion of an organism affects the accuracy of a census and the efficiency of a diver. Conversely, the detectability of an organism varies according to visibility, swell, and the organism's behavior, as well as the diver's ability.

Not surprisingly, the appropriateness of specific sampling techniques has been much disputed among biologists. This preliminary note describes the pattern of dispersion of some common marine macro-invertebrates recently surveyed in the Galápagos archipelago and recommends appropriate survey methods. The special case of the sea cucumber, a commercially valuable species, for which abundance estimates are needed at the population scale, *i.e.* hundreds of km rather than the finer scale appropriate for bio-regional assessment, is also considered.

METHODS

Two survey methods were used: (a) employing a circular quadrat, a diver counted all organisms within a circle determined by a line 5.64 m long fixed at a central point; and (b) using the more traditional line transect, a diver swam along a 50 m or 100 m line and counted all organisms within a meter of one or both sides of the line. The former method covers 100 m^2 and has been used in sea cucumber surveys in the Galápagos; the latter covers 100 m^2, or 200 m^2 if both sides of the line are surveyed, and is used for macro-invertebrate surveys.

A comparison of the two methods was made for surveys at seven sites near western Isabela and Fernandina Islands. The sites near Isabela were: Caleta Iguana, Bahía Elizabeth, Playa Negra and Punta Vicente Roca. Those near Fernandina Island were: Punta Espinosa and Punta Mangle Sur and Norte. At each site, 2-4 (mean 2.3) circular quadrats and 1-5 (mean 3.1) line transects covering 100 m^2 at the same depth were sampled.

Other surveys with 100 m transect lines were done by one of the authors (SAS) at Cabo Douglas, Fernandina Island, El Muñeco, Poza de las Azules (2 depths), Puerto Bravo (2 depths), Piedra Blanca (2 depths) and Caleta Iguana (2 depths) on Isabela Island. All macro-invertebrates were counted in 10 m segments over a total of 200 m^2 in some transects and on 100 m^2 on others. The resultant abundance data for 9 common species were analyzed by a simple test for randomness, the variance-mean ratio (s^2/n, where n is the mean density) [see Sokal and Rohlf 1973] which was calculated for 10 m and 50 m segments for the subset of data available.

To estimate diver efficiency when sighting sea cucumbers, the diver swam in one direction along the line and removed all the individuals encountered. Then he swam back slowly in the opposite direction recording those animals which were missed in the first count. To estimate efficiency at sighting sea-urchins, the diver counted organisms within a square meter. He then removed larger animals which hid other organisms from view and did a recount. To examine the effect of an organism's nocturnal behavior on counts, a transect was delimited in the day and censused soon thereafter. The transect census was repeated several hours later at night. These day-night comparisons were at Punta Moreno (2 sites) and Piedra Blanca on Isabela Island, Las Cuevas and Punta Cormorant on Floreana Island, Site 2 San Cristóbal Island, Bahía Gardner, Española and Isla Bartolomé, near Santiago. The surveys and experiments were done during the period December 2000 to March 2001.

RESULTS

Spatial Dispersion

The results (Table 1) show that all abundant species, with a few exceptions, were aggregated at a spatial scale of 10 m and when data were combined over 50 m segments, aggregation persisted at the larger scale. In the case of *Eucidaris*, s^2/n approached a random distribution at only two sites out of 17. In the case of the sea cucumber, *Stichopus fuscus*, this occurred at one site out of four at a scale of 50 m, and at no sites at a scale of 10 m.

A comparison of density estimates for sea cucumbers at seven sites where both circular quadrats and line transects were done showed that the two methods produced well correlated ($r=0.8$; $P<0.05$) density values. However, the circular quadrat estimates of density were on average 64% higher than the line transect estimates.

Diver Efficiency

Efficiency was estimated for the sea cucumber, *S. fuscus*, at Cabo Douglas on Fernandina, and at Los Cañones, Playa Negra, Punta Moreno and El Muñeco on Isabela along a line transect of 100 m covering an area of 100 m^2. Mean diver efficiency for the five sites was 0.75 (s.e. 0.02) at a mean apparent sea cucumber density of 0.11 (s.e. 0.01) m^{-2}. At all sites, the habitat varied from irregular boulders to blocks, 0.5-3 m diameter.

Efficiency at counting the sea-urchin, *Lytechinus*, was estimated at Poza de los Azules, Isabela Island, where clusters of *Lytechinus* were obscured between groups of *Eucidaris* and boulders to 1 m diameter. In five 1m^2 quadrats randomly placed on the bottom, diver efficiency was estimated to be 0.55 (s.e. 0.07) where the mean apparent density of *Lytechinus* was 6.8 (s.e. 0.9) m^{-2}.

Lastly, diver efficiency at counting *Eucidaris* was estimated at Bahía Elizabeth, Isabela, where the habitat is heterogeneous and where numerous overhangs reduce detectability of organisms. Efficiency in five 1 m^2 quadrats randomly placed on the bottom was 0.57 (s.e. 0.01) at a mean apparent density of 5.2 (s.e. 0.8) m^{-2}.

Nocturnal Behavior

Day-night comparisons of density of macro-invertebrates are given in Table 2. Three species of sea-urchin, *Eucidaris*, *Lytechinus* and *Tripneustes*, showed major changes in density from day to night but with no consistent trend. A fourth species, *Diadema*, showed a large increase at night at two sites. *Stichopus* generally increased in density at night, whereas *Nidorellia* decreased sharply at night. The gastropod, *Hexaplex*, always in low density, showed no consistent trend. For less common species data are too few to show any trend.

DISCUSSION

The pattern of spatial dispersion of a species may be related to the heterogeneity of the habitat or to the behavior of the species. The sea-urchin, *Lytechinus*, a detrital

Table 1. Variance-mean ratios calculated for common macro-invertebrates counted during surveys. N is the number of sites for which data are available. s^2/n is the variance-to-mean ratio, and where s^2/n is close to unity, data are shown separately.

Species	N	Density Range (Nos/10m^2)	s^2/n for 10 m Segments	N	Density Range (Nos/50m^2)	s^2/n for 50 m Segments
Eucidaris thouarsii	16	2.6-112	2.7-26.4	9	13.0-486	2.1-26.8
	2	12.3-28.1	1.3			
Lytechinus semituberculatus	14	2.9-340	2.7-178	6	48.3-162.8	24.9-289
Tripneustes depressus	5	2.8-19.2	2.9-9.4	4	13.8-95.8	6.8-13.3
Nidorellia armata	4	2.4-10.2	3.2-7.0	6	1.8-51.2	1.8-8.1
Stichopus fuscus	7	0.8-7.9	1.5-5.7	3	2.1-6.0	2.1-23.0
				1	7.25	1.3
Stichopus horrens	1	11.8	5.8			
Holothuria atra	1	1.5	4.2			
Holothuria kefersteinii	1	2.9	4.1			
Holothuria fuscocineria	1	0.8	0.95			
Diadema mexicana	1	1.0	8.0-17.5			
Hexaplex princeps	5	0.6-3.8	2.1-7.4	4	3.0-19.0	2.1-5.9

Table 2. Day-night comparisons in density (numbers.10 m^{-2}) at eight sites in the Galápagos. The sites are: 3 on Isla Isabela: PMoreno1; PMoreno2; PBlanca; 2 on Isla Floreana: LCuevas; Cormorant; and at the islands: Española; Cristóbal; Bartolomé. Only significant results (P<0.05) are shown as decreases or increases. Density values are shown for each site. Where no change was noted, the two values are listed.

Species	Site	Increase Night	Decrease Night	No Change
Eucidaris	LCuevas	6 - 25		
	Cristóbal	28 - 58		
	Española	3 - 40		
	Bartolomé	113 - 171		
	Cormorant		20 - 16	
	PMoreno1			5, 3
	PBlanca			12, 15
Lytechinus	Moreno1	0.9 - 28		
	LCuevas	0 - 0.4		
	Española	2.9 - 19.5		
	Bartolomé	4.7 - 9.0		
	PMoreno2		13 - 3	
	Cormorant		30 - 22	
	PBlanca			118, 103
Tripneustes	PMoreno2	15 - 19		
	PMoreno1		7 - 1	
	Bartolomé			6, 4.6
Diadema	Cormorant	0 - 0.3		
	LCuevas	1 - 11		
	Bartolomé			5.7, 3.3
Nidorellia	PMoreno1		2.4 - 0.1	
	PMoreno2		0.6 - 0	
	PBlanca		2.4 - 0	
Stichopus fuscus	PMoreno1	8 - 16		
	PBlanca	2 - 22		
	LCuevas	0.6 - 1.3		
	Cormorant	0 - 0.7		
	PMoreno2		12 - 6	
Stichopus horrens	Bartolomé	0.1 - 11.8		
Holothuria fuscocinerea	Bartolomé	0 - 0.8		
Hexaplex princeps	PMoreno1	0 - 2		
	LCuevas	0.4 - 1.4		
	Cormorant		0.7 - 0	
	Bartolomé			0.8, 1.0

feeder (Wellington 1975), and the sea-cucumbers, *Holothuria* spp., tend to occur in or near sandy patches. Hence, the uneven distribution of sand among rock will cause patchiness in their distribution. The sea-urchins, *Eucidaris* and *Diadema*, are both largely crevice-dependent, although at very high densities *Eucidaris* may be less so, while *Tripneustes* prefers exposed resting sites on open rock. The dispersion pattern of these species is most likely influenced by habitat. The sea-star *Nidorellia* feeds on filamentous algae and sessile invertebrates (Hickman 1997); the sea cucumber *Stichopus* feeds on diatoms and other microphytoplankton, and possibly sessile invertebrates (Aguilar *et al.* 1993); and the gastropod *Hexaplex* preys on barnacles and oysters (Hickman 1997). In all these cases, habitat features affect prey or algal distribution and hence distribution of the predator. However, all of these species except *Hexaplex* are broadcast spawners and aggregate for spawning. Thus spawning behavior may be important as a cause of aggregation.

Estimating the abundance of such species accurately requires understanding of their dispersion pattern. A useful operational rule is that the length of transect chosen for sampling should exceed the scale at which aggregation occurs. Since the majority of these invertebrates are aggregated at a scale of at least 50 m, the minimum length of transect lines should be twice that or about 100 m. Hence, circular quadrats and short transect lines cannot be expected to yield accurate estimates of abundance without a large number of randomized replicates. In the comparison between circular quadrats and 100 m line transects, the correlation was evident, but circular quadrats substantially over-estimated the abundance of *S. fuscus*. The consistently higher values in circular quadrats may be due to the aggregated dispersion of this sea cucumber and the possible bias of researchers toward sea cucumber habitat, or alternatively, avoidance of inferior habitat chosen for placement of the quadrat. This bias is much less likely in the placement of 100 m transect lines. While measures could be taken to avoid bias in the selection of quadrat locations, from a logistical point of view it is probably easier to lay out line transects in an unbiased manner than circular quadrats.

The nocturnal behavior of species which may be cryptic by day can also cause variation in abundance estimates. In the case of the four sea-urchin species described, all are active at night apparently for feeding or for spawning (Wellington 1975). Of these, *Tripneustes* and *Lytechinus* were often seen to crowd together on elevated rocks at night, suggestive of spawning clusters. Hence, it must be expected that day-night differences in density could show large changes in both directions depending on where the transect line lies in relation to elevations where aggregation occurs. *Stichopus fuscus* is also partially cryptic by day, emerging in the late afternoon and evening to feed. Hence, nocturnal counts may be higher, depending on the species' degree of crypsis by day. *Stichopus horrens*, on the other hand, is always cryptic by day and normally seen only at night.

Lastly, there are variations caused by diver experience, diver efficiency, and diver error. In these surveys, we used experienced divers, but inexperienced divers should rapidly gain the experience necessary to recognize the relatively few species involved. However, diver efficiency may vary between habitats. On a few surveys, different divers did the day-night comparisons of transects, so it is possible that some of the differences in observations are due to differences between divers. Meter distances were marked on the transect line, but in all surveys, divers estimated the meter distance from the transect line; this provided another opportunity for diver error. In the current baseline surveys, the accepted protocol was to estimate abundance with minimal disturbance to the habitat. Hence, efficiency values of 0.55-0.75 as recorded here for some echinoderms are inevitable depending on the heterogeneity of the habitat, crowding, or other factors obscuring detection. To meet this source of error, evaluation of efficiency in specific habitats can easily be incorporated into the monitoring protocol.

Estimating the Abundance of Sea Cucumbers

Surveys of sea cucumbers have specific problems because population size estimates are needed at a metapopulation scale in order to fix quotas. This requires a different strategy from that used for other invertebrates for bioregional mapping at a smaller scale. In the Galápagos, *Stichopus* occurs both on rock platforms in shallow bays and on steeply sloping shores. A stratified sampling strategy first used by Bermeo-Sarmiento (1995) in the Galápagos for bays and for steeply sloping coasts has clear advantages. For irregular bottom habitat in bays, the use of long transect lines placed systematically throughout the area under study seems optimal. For similar habitat in Torres Strait, Uthicke (1996) recommended 200´4 m transects with appropriate replication. However, for steeply sloping bottom, the method used in southeast Alaskan fjord habitats is more appropriate. There, transects are set normal to the shore from the intertidal to the lower depth limit. A diver swims down the slope with a meter stick, returning upward at a specified distance from the first transect. From these data, the density of sea cucumbers per km of coast can be calculated. In Alaska, transects are placed on average every 8.5 km over 4000 km of shoreline during surveys extending over three years (Woodby and Larson 1997). Such data, after standardizing for diver efficiency and emergence of sea cucumbers at night, can provide robust biomass estimates for the purpose of setting quotas and estimating parental stock size.

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S. A. Shepherd, South Australian Research and Development Institute, PO Box 120, Henley Beach, 5022, South Australia E-mail:shepherd.scoresby@saugov.sa.gov.au. G. J. Edgar, Tasmanian Aquaculture and Fisheries Institute School of Zoology, Univ. of Tasmania GPO Box 252-05 Hobart Tas 7001Australia E-mail: g.edgar@utas.edu.au. Veronica Toral, Department of Marine Biology and Conservation, Charles Darwin Station, Puerto Ayora, Santa Cruz, Galápagos, Ecuador E-mail: vtoral@fcdarwin.org.ec.